

Essential Amino Acid Supplement Lowers Intrahepatic Lipid Despite Excess Alcohol Consumption

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Abstract

Excess alcohol consumption is a top risk factor for death and disability. Fatty liver will likely develop and the risk of liver disease increases. We have previously demonstrated that an essential amino acid supplement (EAAS) improved protein synthesis and reduced intrahepatic lipid in the elderly. The purpose of this study was to further evaluate the influence of EAAS on intrahepatic lipid (IHL), body composition, and blood lipids in individuals with mild to moderate alcohol use disorder (AUD). Following consent, determination of eligibility, and medical screening, 25 participants (18 males at 38 ± 15 years/age and 7 females at 34 ± 18 years/age) were enrolled and randomly assigned to one of two dosages: a low dose (LD: 8 grams of EAAS twice/day (BID)) or high dose (HD: 13 grams of EAAS BID). Five of the twenty five enrolled participants dropped out of the intervention. Both groups consumed the supplement BID for 4 weeks. Pre- and post-EAAS administration, IHL was determined using magnetic resonance imaging/spectroscopy, body composition was analyzed using dual energy x-ray absorptiometry, and blood parameters were measured by LabCorp. T-tests were used for statistical analysis and considered significant at $P < 0.05$. While there was no significant change in IHL in the LD group, there was a significant 23% reduction in IHL in the HD group ($p = 0.02$). Fat mass, lean tissue mass, bone mineral content, and blood lipids were not altered. Post-EAAS phosphatidylethanol was elevated and remained unchanged in LD at 407 ± 141 ng/ml and HD at 429 ± 196 ng/ml, indicating chronic and excess alcohol consumption. Based on these results, we conclude that 13 grams of proprietary EAAS consumed BID lowers IHL in individuals with mild to moderate AUD.

Keywords: amino acids; liver; alcohol

Introduction

Alcohol use disorder (AUD) is a leading risk factor for death and disability and is responsible for 69 million Disability Adjusted Life Years (DALYs) [1]. Chronic alcohol use induces hepatic steatosis in 90-95% of individuals; liver pathology advances to cirrhosis in approximately 8-20% of individuals with AUD and represents one of the most important clinical problems associated with AUD [2]. Ameliorating the metabolic consequences of AUD requires more than abstinence and good overall nutrition, as the majority of individuals with AUD continue to drink alcohol, increasing their risk for liver disease [3]. This clinical scenario is not dissimilar from other diseases involving lipotoxicity, in which unhealthy human behaviors require a combination of pharmaceutical, surgical, or nutritional approaches [4].

Many individuals with AUD are malnourished, and the degree of alcoholic liver disease severity correlates with the degree of malnutrition [5]. The dietary intake of protein and micronutrients often fail to meet recommended levels, even during professional, supervised recovery from AUD [6,7]. In these circumstances, a nutritional supplement designed to address the specific metabolic issues associated with the condition may provide unique benefits. A variety of nutritional supplements targeting some aspect of the AUD responses are available or have been proposed [8-20]. However, none have corrected the disruptions in macronutrient metabolism that lead to hepatic steatosis [11].

The beneficial influence of unique essential amino acids (EAAs) on the stimulation of protein synthesis [12-14] and reduction in hepatic steatosis in older adults without AUD has been demonstrated [13]. Ingestion of an EAA-based formula resulted in a 50% reduction in liver fat after only four weeks of therapy [13]. Individuals receiving the EAA formula consumed a balanced diet containing at least the minimum RDA for protein [15]. Still, the EAAs exerted a profound effect on liver health. The ingestion of an EAA-based formula promotes higher plasma EAA concentrations when compared to isocaloric ingestion of intact protein, corresponding with

higher net protein balance [16]. The etiology of hepatic steatosis is not identical among those at risk for metabolic disease and those who present with AUD [17]. Hepatic steatosis can occur in part because of a limitation in mitochondrial function [18], and thus impaired fatty acid oxidative capacity [19]. Impaired fatty acid oxidation results in the channeling of fatty acids into triglyceride synthesis. Based on the importance of EAAs in the promotion of mitochondrial protein synthesis in the liver [20], we hypothesized that a nutritional formula enriched with either 8 or 13 grams of EAAs would reduce excess intrahepatic lipid in persons with AUD, even when alcohol consumption remained unchanged. If so, the status quo for treatment of individuals with AUD may be modified to induce improved clinical outcomes in this segment of the population.

2. Materials and Methods

2.1. Recruitment

We utilized newspaper advertisements and posted fliers and employed a secured, telephone answering service, allowing private telephone evaluations of individual eligibility. Once it was established that the individual was a potential participant via telephone screening, we scheduled an actual screening visit at the Clinical Research and Imaging Facility (CRIF), located within the Murie Building on the UAF campus [21]. The location of the CRIF is well suited for work with volunteers from the community as there are dedicated parking spaces, and there is semi-private access with little to no student, faculty, or staff traffic. These were especially important factors in minimizing any stigmas that might be associated with participation of volunteers in the proposed study. The screening visit included the informed consent process, blood work and health history. Based on the eligibility criteria for mild to moderate AUD, participants were either eligible to participate or referred to their primary care physician for follow up medical care. The project (Nutrient Formulation for Liver Health: 986801-17) and all related documents were approved on December 15, 2016 by the University of Alaska Fairbanks Institutional Review Board.

We chose to restrict our age range, as older individuals could have promoted significant variability in baseline data due to metabolic changes that occur with aging [22]. When an individual was determined to be potentially eligible via telephone interview, a screening visit was scheduled to perform a medical history and physical exam. All participants were required to have transportation to the site clinic for the screening, consent process, testing sessions, weekly checkups, and pickup/return of EAAS. A capability for understanding and providing informed consent was necessary for all participants. After the screening visit, the study physician reviewed the exams and blood sample analysis, and all participants were advised of their health and eligibility status.

2.2. Exclusion Criteria

Any person with a pacemaker or other implanted metal, insulin-dependent diabetes, or chronic inflammatory condition were excluded. Individuals taking any type of oral contraceptive or any medication or supplement affecting glucose metabolism were excluded. Individuals with active cancers or malignancies were ineligible, as were those taking corticosteroids by mouth, injection or trans-dermally. If the study physician concluded that any medical condition or current medication to represent an unacceptable risk, those individuals were excluded.

2.3. Study In

Once eligibility was established, participants were randomized to a low dose (LD) (8 grams of EAAs (twice/day (BID)) or high dose (HD) (13 grams of EAAs BID) supplementation and asked to undergo two testing sessions in conjunction with the 4-week supplementation phase. In each of the testing sessions (ie., pre-supplementation and post-supplementation), participants underwent magnetic resonance imaging (MRI)/magnetic resonance spectroscopy (MRS) scans, and dual energy x-ray absorptiometry (DXA) scans in the CRIF, and blood sampling at LabCorp (Figure 1). During the 4-week supplementation period, participants were requested to visit the CRIF at weekly intervals to retrieve their EAAS, evaluate their compliance to the protocol, and measure their weight. Compliance to the paradigm for EAAS supplementation was performed

by the measurement of weight difference in the EAAS product provided and returned each week.

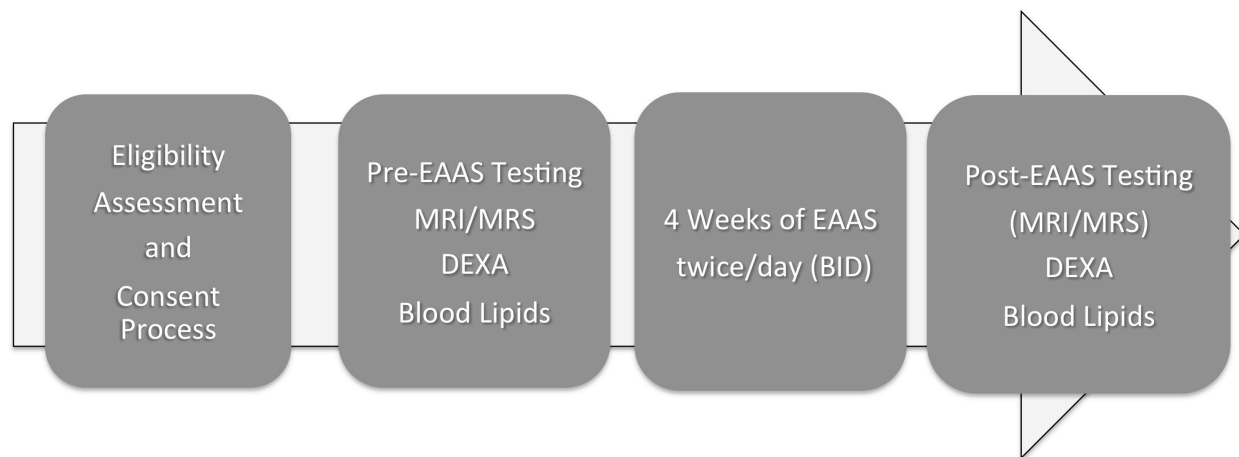


Figure 1. Study timeline

2.4. Intrahepatic Lipid

We utilized the Toshiba 1.5T Excelart/Vantage with a 1.4 m magnet and a 65.6 aperture, and IHL measurements were performed in the middle right lobe [23] (Figure 2). The scans were localized to the same area of the liver using anatomical orientation of the hepatic blood flow and ribs, so that approximately the same area of liver was scanned during each testing session. After a T1 scan for anatomical structures, a voxel (~30 mm × 30 mm × 30 mm) was chosen at a location free from large vessels. An optimized spectroscopy sequence was run 256 times without respiratory gating. These spectra provided an average lipid concentration measurement

over the mid-right lobe. Spectra were manually phased, and final analysis was then performed with jMRUI (Figure 3).

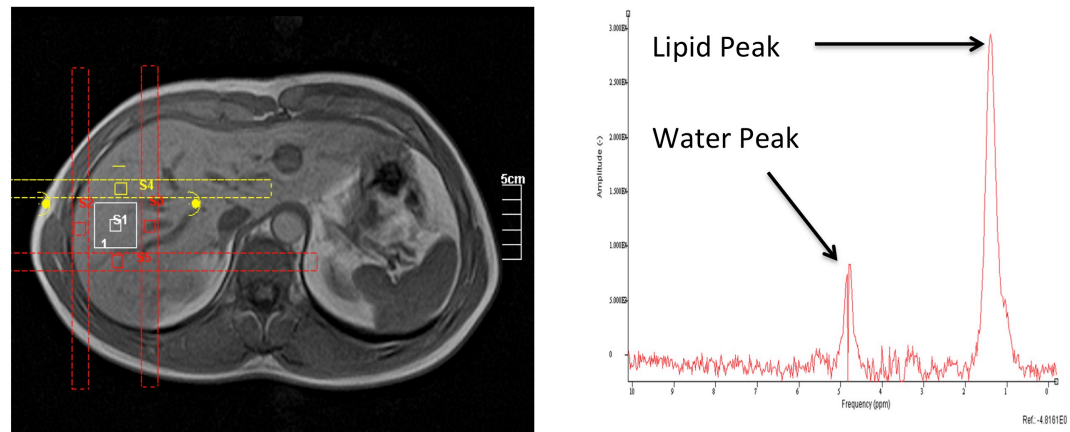


Figure 2. MRI of liver detailing location of voxel and example of 1-H spectroscopy derived measurement of intrahepatic lipid.

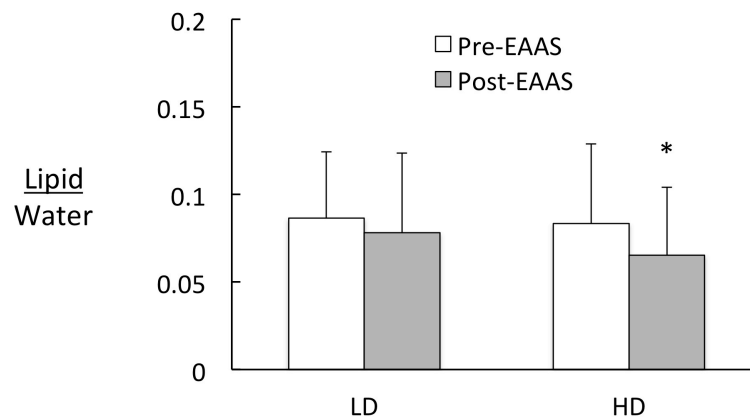


Figure 3. Intrahepatic lipid in low dose (LD) and high dose (HD) pre- and post-essential amino acid supplement (EAAS). *Represents a significant difference between pre- and post-EAAS ($P=0.02$).

2.5. Body Weight and Composition

Total body mass was measured using an electronic scale (Ohaus Corp, USA). A General Electric Lunar iDXA was used to determine fat mass, lean tissue mass, and bone mineral content ¹.

2.6. Blood Measurements

Blood sampling and analysis was performed by LabCorp (1626, 30th Avenue, Fairbanks, AK). LabCorp is staffed with licensed healthcare professionals, accredited by the College of American Pathologists, and licensed through the Clinical Laboratory Improvement Amendment (CLIA). In this study, serum lipid, liver, and metabolic panel analysis were included. The lipid panel included total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), and triglycerides.

The liver panel consisted of albumin, alanine transaminase (ALT), aspartate transaminase (AST), bilirubin (total and direct), and total protein. The metabolic panel included blood urea nitrogen (BUN), calcium, carbon dioxide, chloride, creatinine, glucose, potassium, and sodium. Whole blood phosphatidylethanol (Peth) was measured to ascertain the level of alcohol consumption.

2.7. Statistical analysis

Data were analyzed using Microsoft Excel, iDXA Encore, Osirix, and Prism 5 software. Data are presented as means \pm SD. Two sample homoscedastic t-tests were used to evaluate potential differences between groups. Paired t-tests were utilized to compare differences in pre-supplementation and post-supplementation within groups.

3. Results

Research Participants. We enrolled 25 research participants (18 males and 7 females) with mild to moderate AUD for this study. Seventeen individuals completed all aspects of the study; five dropped out and three participants failed to get their post-supplementation blood sampling. Based on EAAS weigh back data, the average daily compliance to EAAS was 85 \pm 15% and 83 \pm 8% in LD and HD groups, respectively. The average weight, body mass index, and body composition was similar between groups and did not change with EAAS.

Intrahepatic Lipid. IHL was elevated at baseline in both groups [25] and decreased by 23% in the HD group with EAAS (Figure 3). The significant reduction in IHL represented approximately half of the reduction needed to return IHL to normal levels [25,26].

Blood parameters. Total cholesterol, LDL-cholesterol, VLDL-cholesterol, HDL-cholesterol, and triglycerides were all within normal limits and did not differ between groups and did not change with EAAS (Table 1). Except for blood Peth, all other blood parameters were also within normal limits and were not altered by EAAS, indicating the lack of any undesirable effects of EAAS on lipid, liver or metabolic function (Table 1). Blood Peth levels were elevated but not different between groups and were not altered from pre- to post-supplementation in either

group. Blood Peth levels above 400 ng/dl confirmed alcohol misuse in all participants (Table 1) [27].

Table 1. Clinical Characteristics	Pre-EAAS LD	Post-EAAS LD	Pre-EAAS HD	Post-EAAS HD	NORMAL RANGE
Sex (F/M)	4/4	4/4	2/7	2/7	-
Weight (kg)	81±11	81±10	74±11	74±12	-
Body Mass Index (kg/m ²)	26±3	26±1	25±4	25±4	18.5-25.9
Fat Mass (kg)	20±7	19±7	19±7	19±7	-
Lean Tissue Mass (kg)	57±11	58±11	51±8	51±8	-
Total Cholesterol (mg/dL)	187±26	176±21	184±32	186±35	100-199
LDL-cholesterol (mg/dL)	100±22	87±21	105±17	103±21	0-99
VLDL-cholesterol (mg/dL)	23±13	35±24	22±22	23±20	5-40
HDL-cholesterol (mg/dL)	57±13	55±14	58±12	60±12	>39
Triglycerides (mg/dL)	152±113	175±115	107±13	117±98	0-149
Albumin (g/dL)	4.5±0.4	4.4±0.3	4.5±0.2	4.6±0.1	3.5-5.5
ALT (IU/L)	25±11	18±7	20±10	18±9	0-44
AST (IU/L)	23±7	19±5	22±6	29±21	0-40
Bilirubin – total (mg/dL)	0.5±0.3	0.5±0.3	0.7±0.6	0.6±0.2	0.0-1.2
Bilirubin – direct (mg/dL)	0.1±0.1	0.1±0.1	0.2±0.1	0.2±0.0	0.0-0.4
Protein - total (g/dL)	7.1±0.4	6.7±0.4	7.0±0.4	6.8±0.4	6.0-8.5
BUN (mg/dL)	14±4	14±4	16±5	16±4	6-24
Calcium (mg/dL)	9.4±0.2	9.5±0.2	9.5±0.3	9.4±0.3	8.7-10.2
Carbon Dioxide (mmol/L)	24±1	24±1	24±2	25±1	20-29
Chloride (mmol/L)	102±1	100±1	101±2	101±2	96-106
Creatinine (mg/dL)	0.9±0.1	0.9±0.1	0.9±0.2	0.9±0.1	0.76-1.27
Glucose (mg/dL)	90±7	90±5	85±5	86±7	65-99
Potassium (mmol/L)	4.5±0.4	4.3±0.2	4.3±0.2	4.4±0.2	3.5-5.2
Sodium (mmol/L)	141±1	140±2	141±1	142±1	134-144
Peth (ng/mL)	407±141	429±196	429±196	422±224	<20

4. Discussion

The primary focus of this investigation was whether EAAS BID would decrease IHL in individuals with mild to moderate AUD. We have now demonstrated that 13 grams of EAAS provided BID significantly reduced IHL without manipulation of dietary intake, change in habitual alcohol consumption, or any form of behavioral modification. On the other hand, 8 grams of EAAS BID did not influence IHL. Given the overall normal ranges for blood parameters except for Peth, it was not surprising that here were no changes in circulating lipid, liver, or metabolic blood parameters in either group. Future studies in a larger cohort with a longer intervention paradigm may be necessary to evaluate whether lower doses of EAAS could IHL.

Several studies have posited the beneficial influence of essential amino acids on the mitigation of hepatic steatosis [28-31]. To date, this work has focused on nonalcoholic hepatic steatosis, which also describes excessive accumulation of IHL similar to alcoholic hepatic steatosis [32]. Recommendations suggest that a diet containing foods with more favorable glycemic indexes and energy values coupled with reductions in saturated fat intake should be combined with increased exercise and weight reduction to lower IHL in those with non-alcoholic hepatic steatosis [33]. Unfortunately, adherence to behavioral modification and/or lifestyle intervention has proven extremely difficult [34] for many individuals with hepatic steatosis; regardless of the underlying pathology.

The mechanisms responsible for EAAS-mediated improvements in IHL in the current study may be linked to their influence on mitochondrial biogenesis [35] and/or the complex modulation of AMPK α , mTOR, sirtuin-1, and PPAR- γ , all of which regulate pathways of fatty acid kinetics [31]. The provision of EAAS has also been demonstrated to reduce insulin resistance, a common factor implicated in the excessive deposition of lipids in the liver [36]. In the current study, subjects had normal blood glucose concentrations and thus mitigation of insulin resistance was unlikely to have been a significant factor in reducing IHL. Whereas a nonspecific increase in overall dietary protein intake might introduce unnecessary nonessential amino acids linked to excess ammonia and urea production [37], provision of the higher dose of EAAS BID may improve IHL through augmentation of mitochondrial volume and turnover [38].

Suppression of mTORC1 via alcohol intake presents a completely different physiological circumstance [39] than the association between BCAAs and increased mTORC1 in obesity [40]. Given that mTORC1 is vitally important in the stimulation of mitochondrial biogenesis and the corresponding increment in oxidative metabolism needed to support anabolism of metabolic machinery [41], it is not surprising that the provision of EAAS provided beneficial alterations in IHL. While the risks for development of metabolic syndrome in individuals with AUD is two-fold higher than the rest of the population [42], this is likely due to interactions between dietary

intake, lack of physical activity and alcohol intake [43]. The serum lipids in our participants while at the top end of the normal range were still in fact within normal limits. Therefore, it was relatively unlikely for cholesterol or triglycerides to decrease in this cohort.

Regardless of the lack of therapeutic benefit of EAAS on serum lipids that were not elevated by established clinical standards, the beneficial reduction in IHL was significant. Other studies have demonstrated similar benefits in individuals at risk for metabolic diseases [31,44], but this is the first study to our knowledge that has established a link between EAAS and the reduction of IHL in individuals with AUD. It is our assertion that EAAS may have likely improved cytosolic concentrations of amino acids in the liver, which positively altered mitochondrial protein synthesis as previously demonstrated in pre-clinical studies [20]. Combined with the impact of EAAS on transcription via their influence on mTOR [45], these molecular avenues may have allowed EAAS to exert their beneficial influence on mitochondrial biogenesis.

We recognize that the lack of strict dietary control and/or management of physical activity patterns represent limitations of our study design as both factors may affect IHL [46,47]. Nonetheless, the BMI of our participants indicated that obesity was unlikely to be a contributing factor in the accumulation of excess IHL and the exemption of individuals with diabetes eliminated the influence of that particular disease process on liver metabolism. Instead, we chose to focus on the efficacy of a simple nutritional therapy (ie., EAAS) on IHL in the context of alcohol misuse (as supported by elevated and stable Peth levels) [48]. This strategy was consistent with our intention to minimize the complexity of the intervention, maximize EAAS compliance, and improve the potential for practical applications based on solid clinical evidence.

5. Conclusions

Finally, why provide a supplement that could somewhat offset the deleterious influence of alcohol on liver metabolism? The answer to this important question lies in the fact that less than 7% of individuals with AUD will actually seek professional treatment [49], even though early mitigation of steatosis may delay the progression of alcoholic liver disease [50]. As indicated by

aminotransferase levels within normal limits in our own participants, hepatic steatosis will likely exist prior to the ability to detect liver damage via blood sampling/evaluation [51,52]. With overwhelming evidence that very few adults access professional treatment for AUD [53], therapeutic alternatives are needed to decrease the pernicious influence of alcohol induced hepatic steatosis on health outcomes. Otherwise, the complex etiology of alcohol-related liver disease; including poor nutritional status [54], impairments in fatty acid oxidation [52], and perturbations in the NADH:NAD⁺ ratio will continue to worsen the condition of the liver [55]. Future clinical studies should be directed toward a larger cohort with more pronounced dyslipidemia and longer EAAS interventions.

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Conflicts of Interest: Drs. Coker and Wolfe are Managing Partners and Co-Owners of Essential Blends, LLC that has received funding through the Small Business Innovations in Research from the National Institutes of Health to develop clinical nutrition products. We declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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